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A Novel Pressure Indicator for Continuous Flow PCR Chip Using Micro Molded PDMS Pillar Arrays

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ABSTRACT

DNA amplification is one of the most routine experiments carried out in biological laboratories. Continuous flow PCR chip releases biologists from their laborious exercises. The use of such chip is, however, hindered by costly expense of the syringe pump, which is used to maintain a constant flow rate. In this paper, we demonstrate a novel pressure indicator which makes up an in-line flow sensor in a continuous flow PCR chip. The pressure of PCR channel is achieved from pattern change in fabricated microstructures, and converted to volume flow rate. A polymeric PCR chip with such pressure indicators is presented. With a much less expense as compared to its conventional peers, this indicator has a wide potential for the use in the laboratories which runs daily activities like sequencing or mutagenesis.

INTRODUCTION

Continuous flow PCR (polymerase chain reaction) chip, first reported by Kopp [1], has received considerable attention in recent years. This chip has three well-defined zones kept at 95°, 77°, and 60°C using thermostated copper blocks with PID temperature control. The PCR sample and buffer solution are hydrostatically pumped through a single channel etched into the glass chip using two precision syringe pumps. The channel passing through the three temperature zones defines the thermal cycling process. The thermal cycles are realized by keeping temperatures constant over time at different locations in the system and moving the sample through the individual temperature zones. Given that the time delay for the sample to reach a new temperature solely depends on the time needed to transport the sample into the appropriate temperature zone by heating the fluid element in the channel, the volume flow rate is crucial for the time-space conversion. It is to ensure the sample can perform cycles of denaturation, annealing and extension successively. A precision syringe pump is currently in use for most cases in the above system. However, its costly expense, usually over one thousand dollars, keeps it from the use by a wide public in their daily PCR experiments. The syringe pump also raises a problem in system integration because it needs to be close to the PCR chip, or the long flexible transfer tube will somehow compromise accuracy of volume flow rate. Moreover, the syringe pump lacks capability for responding to possible variation in micro channels. Thus, an economic in-line flow meter is urgently required.

Many engineered flow sensors for microfluidic systems arise right at the moment to provide feedback based on a number of sensing principles, including differential pressure [2], dispersion of a thermal pulse [3], injection and subsequent detection of a charge pulse carried along in the fluid as ionic species [4], and many etc. Despite significant advances made by these devices, however, most of them are made from silicon material and need electronics plating for pulse generation or read-out, which involves complex fabrication processes and expertise.

Therefore, we plan to develop a PDMS(polydimethylsiloxane)-based continuous flow PCR chip with an integrated flow sensor. With most components made from PDMS, the cost and fabrication complexity of the proposed chip is greatly reduced as compared to any of its current

peer. In this chip, the precision syringe pump can be replaced by a less expensive liquid pump without losing control of volume flow rate. It makes the chip virtually accessible to laboratories of all sizes for their daily PCR experiments.

In this paper, we demonstrate the polymeric pressure indicators integrated within the proposed PCR chip, which make up an in-line flow sensor. Pressure in the PCR channel is derived from pattern change of the microstructures that has been replicated onto the polymeric surface. The flow rate is derived from the differential pressure, thereby ensuring a required time delay of PCR sample in each thermal zone even if there is an unexpected disturbance.

INTEGRATION OF PRESSURE INDICATORS INTO PCR CHIP

The proposed PCR chip is made up of a base glass slide and a double-side molded PDMS substrate (Figure 1). The three temperature zones are defined by thermal resistors on the base glass slide, and the serpentine PCR channel is formed on the PDMS substrate. The dimensions of the channel are designed in such a manner that the PCR sample can experience required thermal cycles repeatedly with a certain constant flow rate. As shown in the figure, there are two through holes on the PDMS substrate for inlet and outlet access. Two pressure indicators locate between the inlet hole and the PCR channel. Each indicator has a square cavity on the bottom side, covering by a thin membrane on the top, with array of microstructures thereon (Figure 2). These two indicators are connected by a channel with a reduced cross section, serving as a hydraulic resistor. Pressures measured at the indicators are the complementary parameters to compute the applied hydraulic power of the liquid pump. The differential value is also used to calculate the volume flow rate in the PCR channels. The flow sensing principle will be elaborated later in this paper.

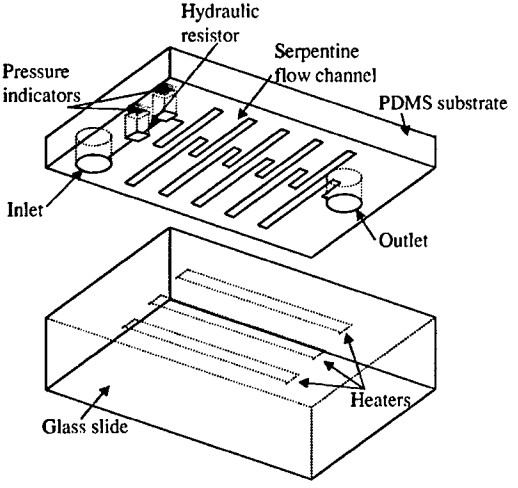

Figure 1. Schematics of the PDMS-based continuous flow chip with an in-line flow sensor.

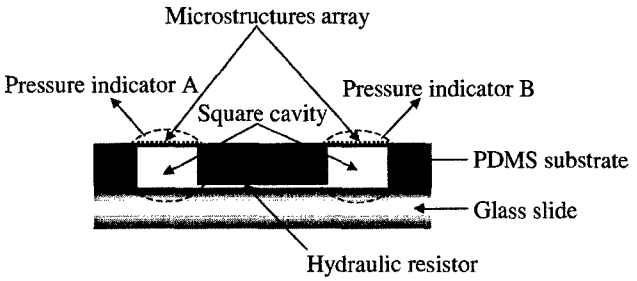

Figure 2. Cross section of PDMS flow sensor. The two pressure indicators are connected by a channel with a reduced cross section, serving as the hydraulic resistor. The differential pressure between A and B is used to calculate the flow rate.

FABRICATION

In this work, a double-side micro molding process was performed to fabricate the PCR channel and the pressure indicators in one-step replication (Figure 3). The bottom master template was prepared by using a laser-assist fabrication technique, which we have introduced elsewhere [5]. Multilayered SU-8 microstructures were fabricated on a glass slide, making up the PCR channel and the hydraulic resistor. To guarantee that the static pressure instead of the dynamic pressure is measured, the fluid velocity in the resistor must be much higher than at the points where pressure is measured. Therefore, the SU-8 structure for the square cavity is much higher than that for the hydraulic resistor. In this work, a silicon bottom template was also manufactured through a standard microfabrication process. The top master template was fabricated from a silicon wafer by using a standard microfabrication process, with 5 μm holes array on the surface. The two templates (top and bottom) were then placed face to face and aligned to each other, with PDMS prepolymer (Sylgard® 184, mixed 1:10) sandwiched in between, followed by a curing process at an elevated temperature in which the polymer chemistry crosslinks. After completion of thermal curing, the templates were removed carefully. A PDMS substrate containing the PCR channel and the pressure indicators was thus made.

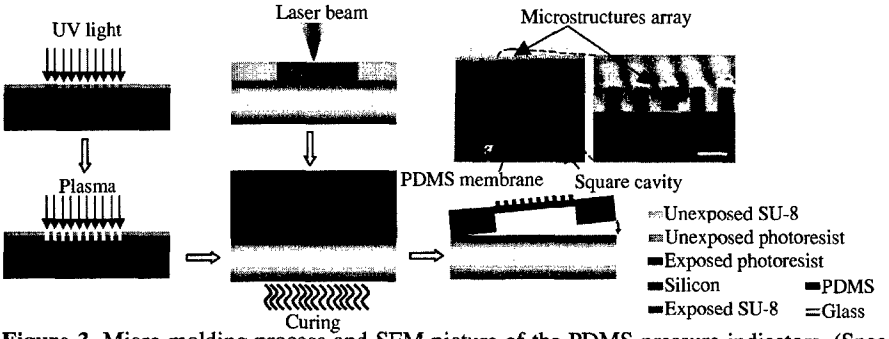

Figure 3. Micro molding process and SEM picture of the PDMS pressure indicators. (Space bar indicates 10 μm)

A glass slide coated with photoresist was patterned and developed, followed by a lift off process in which three thermal resistors was deposited thereon, defining the temperature zones. The fabricated PDMS substrate and the glass slide were then put into contact. The hydrophobicity of the fresh PDMS surface ensures the self-sealing of the two surfaces. The proposed PCR chip was thus manufactured with additional connections to fluidic and electric supplies. The pattern change of the microstructures in the center of the square membrane was observed through an optical microscope and the pressure was achieved as described below.

FLOW SENSING PRINCIPLE

Differential pressure v.s. Flow rate

The flow rate sensing principle of the proposed pressure indicators is to measure the pressure drop over a hydraulic resistance. A conversion of kinetic energy to potential energy can be accomplished by leading the flow through a converging nozzle such that the speed is increased locally. The fluid speed can be derived by measuring the static pressure and using the relation between pressure and velocity according to Bernoulli's principle.

Because of the small channel size and, consequently, the small hydraulic diameter as well as a small Reynolds number, liquid flows in micro channels are in most cases laminar. According to the theoretical models based on the Bernoulli equation, the pressure drop Δp along a micro channel can be expressed as [6]:

$$\Delta p = f \frac{\rho u^2}{2D_h} L \tag{1}$$

where f is friction coefficient, ρ the fluid density, the linear flow velocity u, L the channel length and D_h the hydraulic diameter. Given the definition of Reynolds number, equation (1) can be rewritten as:

$$\Delta p = \mathrm{Re}f \frac{\eta L}{2D_h^2} u \tag{2}$$

where η is the dynamic viscosity of the fluid and the product of Ref can be determined empirically from channels with various cross sections.

Among these parameters, the hydraulic diameter is well-known for approximating the flows through fluidic channels with various geometries. It is given by:

$$D_h = \frac{4A}{P_{wet}} \tag{3}$$

where A is the cross section area and P_{wet} the wetted perimeter. In this paper, we assume that the PCR channel is completely filled by sample fluid after surface treatment. The wetted perimeter is simply the perimeter of the channel and the area is the cross section area of the channel. Given that the channel was replicated from the bottom master template made of SU-8 or silicon, the cross section is either a rectangular (length: a; width: b) or a trapezoid (bases: a, b; height: h), where the hydraulic diameters are given by:

$$D_h = \frac{2ab}{a+b} \qquad \text{(Rectangular)} \tag{4}$$

$$D_h = \frac{2h}{1+\frac{2h}{a+b}\sqrt{1+\left(\frac{a-b}{2h}\right)^2}} \qquad \text{(Trapezoid)} \qquad (5)$$

Inserting (4) or (5) into (2), it can be seen with a defined channel cross section, the pressure drop is a linear function of the volume flow rate ϕ, which is the product of the flow velocity and the cross section:

$$\Delta p = \frac{\eta}{8}\left[Ref \cdot \frac{(a+b)^2}{a^3b^3}L\right]\phi \qquad \text{(Rectangular)} \qquad (6)$$

$$\Delta p = \frac{\eta}{8}\left[Ref \cdot \frac{\left(1+\frac{2h\sqrt{1+(\frac{a-b}{2h})^2}}{a+b}\right)^2}{(a+b)h^3}L\right]\phi \qquad \text{(Trapezoid)} \qquad (7)$$

Differential pressure v.s. Strain

The PDMS membrane is deformed by the difference between the fluidic pressure from the bottom (P_l) and the atmosphere pressure from the top (P_{atm}) (Figure 4). This difference can be represented by the induced strain in the top surface. In order to achieve the strain/pressure relationship, a function form of the deflected membrane was assumed. The total strain energy was minimized to find the load-deflection behavior. In the square membrane, the relationship has the form of [7]:

$$P_l - P_{atm} = \frac{3.04h}{l^2}\sigma_0 d + \frac{1.37(1.075-0.292\gamma)h}{l^4}\frac{E}{1-\gamma}d^3 \qquad (8)$$

where d is the central deflection, l the half-edge length of the membrane, h membrane thickness, E the Young's modulus, γ the in-plane Poisson's Ratio and σ_0 the residual stress.

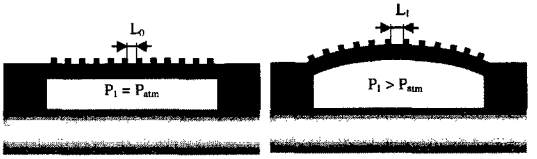

Figure 4. Pressure in PCR channel induces deformation of PDMS membrane, which can be represented by pattern change in the microstructures.

For small deflection, a linear relationship can be used:

$$\frac{L_1 - L_0}{L_0} = \varepsilon_{xx} = \varepsilon_{yy} = \frac{0.51(1-\gamma^2)(P_l - P_{atm})l^4}{Eh^2} \qquad (9)$$

where $L_1$and L_0 are the spacings of the central microstructures from stressed and unstressed membrane, respectively.

For deflection larger than half thickness of the membrane, the middle surface becomes appreciably strained and the in-plane stress cannot be ignored. It will stiffen the membrane material, and drive the problem into geometric nonlinearities and possibly material nonlinearities (plasticity). For simplification, in this work, the polymeric membrane was manufactured thick

enough. The induced deflection was examined using an optical microscope to ensure it is no more than half of the membrane thickness.

PATTERN CHANGE

The pattern changes in the polymeric microstructures were achieved and digitalized. A representative set is shown in Figure 5. The membrane without fluid flowing below suffers no deflection. The spacing of the neighboring structures was taken as a reference. After the fluid was led into the PCR channel, both pressure indicators were deflected. Each of them has an increased spacing upon the deflection. Inserting the spacing into (9) along with the reference, the pressures at the points of both indicators were achieved. The volume flow rate is hence derived by putting the difference of the pressures into (6) or (7), depending on the material used for the bottom master template. Given a predetermined value of the flow rate for the PCR chip, a feedback loop was set to adjust the output of the liquid pump. A precise control of flow rate was thus accomplished.

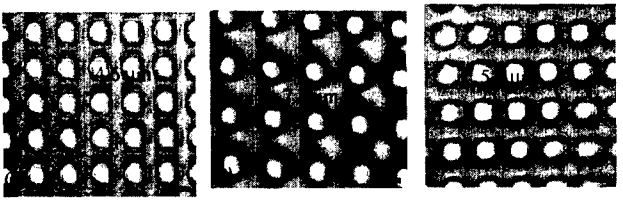

Figure 5. Microstructures array observed from: (a) the non-deflected membrane; the deflected pressure indicators before (b) and after (c) the fluid flows through the hydraulic resistor. (Space bars indicate 5 μm)

CONCLUSION

In this paper, we demonstrate a novel pressure indicator which makes up an in-line flow sensor for continuous flow PCR chips. The volume flow rate can be in-situ monitored using such pressure indicators. With the flexible fabrication and low cost, the pressure indicator can be widely applied in biological laboratories in their daily experiments.

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